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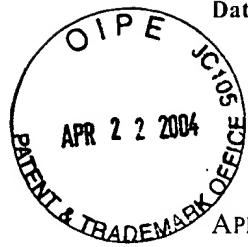
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Peled et al.
SERIAL NUMBER: 09/463,320 EXAMINER: Michail A. Belyavskyi, Ph.D.
FILING DATE: January 22, 2000 ART UNIT: 1644
FOR: METHODS OF CONTROLLING PROLIFERATION AND DIFFERENTIATION OF STEM
AND PROGENITOR CELLS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. EITAN FIBACH UNDER 37 C.F.R. §1.132

1. I am a co-inventor, together with Tony Peled and Avi Treves, of the subject matter claimed in the above-referenced U.S. patent application.
2. I received my Ph. D. degree from the Weizmann Institute of Science in Rehovot, worked as a post-doctoral fellow at the Cancer Research Center, College of Physicians and Surgeons of Columbia University, New York, NY, USA, and was a visiting scientist at the Laboratory of Chemical Biology, NIDDK, National Institutes of Health, Bethesda, MD, USA.
3. I am presently employed as researcher at the Hadassah University Hospital, Department of Hematology, where I am a full professor. My research focuses on the development of hematopoietic cells. Since the beginning of my career, I have published more than 150 scientific articles, more than 40 on various aspects of HL-60 cells, in highly regarded journals and books, and have presented my achievements at many international scientific conferences. I am a member of American Society of Hematology and The International Society of Experimental Hematology. I have served on the editorial board of the journal "Experimental Hematology".
4. I have reviewed the Office Action dated February 9, 2004. I understand that claims

1-2, 4-13, 15, 37-45 and 47 claims 1-2, 4-5, 7-13, 15, 37, 39, 42-45 and 47-57 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Moore et al., Blood Cells, 20: 468-48, 1994 (“Moore”); or De Bruyn et al., Stem Cells 13: 281-288, 1995 (“De Bruyn”), each in view of Cicuttine et al. Blood 80: 102-112 (1992) (“Cicuttine”) and of Percival , J. Nutrition 122: 2424-2429 (1992) (“Percival I”).

5. I have reviewed the present application in conjunction with the Moore, De Bruyn, Cicuttine and Percival I references.
6. The claims as amended require providing hematopoietic cells *ex vivo* with a transition metal chelator having an affinity for copper and proliferation conditions which result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state.
7. There has been a long-felt but unsolved need for methods that permit *ex vivo* expansion of hematopoietic cells (and particularly subpopulations of clonogenic stem and progenitor populations therein). Hematopoietic cells are currently routinely expanded from bone marrow or stored umbilical-cord blood to reconstitute the immune systems of patients with leukemia or other hematologic cancers. Traditional methods of hematopoietic cell expansion have typically not yielded sufficient quantities of hematopoietic cells to treat adult patients. The methods of the present invention solves this long-felt need by providing greater expansion of hematopoietic cell populations and specifically of cells with engraftment ability such as the stem and progenitor subpopulations (e.g. CD34⁺ or AC133 cells and subsets) which increases the effectiveness of short-term and long-term engraftment when the expanded population is transplanted into patients.
8. The present invention teaches that providing hematopoietic cells with a transition metal chelator in combination with early acting cytokines results in not only an inhibition of differentiation and increased cell proliferation but greatly increases the expansion of a subpopulation of clonogenic cells (CFUc) (representative of the stem and progenitor populations) and maintains undifferentiated cells in their

undifferentiated state (*See*, specification at Example 1, page 34, line 8 – page 42, line 33, Table 1 and Figures 1-4).

9. Specifically, the addition of a transition metal chelator, such as TEPA, to cultures containing an early acting cytokine, such as IL-3, surprisingly resulted in a two-fold increase in total cells, four-fold increase in percentage of CD34⁺ cells present in the total cell population and, most strikingly, a five-fold increase in clonogenic cell (CFUc) expansion (*See*, specification Table 1 at page 36, comparing rows 4 and 5). These results demonstrate that providing hematopoietic cells with a transition metal chelator led to excellent expansion of hematopoietic cells. Moreover, these results show the superior and preferential expansion of progenitor subsets such as clonogenic cells (CFUc) and CD34⁺ cells. The fold expansion of progenitor cell subsets is higher than the fold expansion of total nuclear cells. Thus, progenitor cell frequency increased among the entire cell population. The enrichment and expansion of this subpopulation of hematopoietic cells, i.e., stem or progenitor cells, is critical to improving short-term and long-term engraftment in hematopoietic cell transplantation and adoptive immunotherapy.
Moreover, the results in Example 1 also surprisingly demonstrate that a transition metal chelator with affinity for copper (e.g., TEPA) greatly improved clonability in long term cultures, and in fact, the clonability of long term cultures surpass that of cells in short term cultures (*See*, specification at Example 1, page 37, lines 4-7 and Figures 3-4). This data displays the superior properties of the claimed methods, since it is essential that self-renewal be maximally prolonged in order to achieve maximal *ex vivo* expansion of hematopoietic cells.
10. Recent studies, phase I human clinical trials, have confirmed and expanded on the results disclosed in Example 1 of the present invention. The trial comprised four human patients (3 Males, 1 Female) with varying diagnoses and ranging in age from 10-24 and in weight from 50-77 kg. In the trial, hematopoietic cells from donors were treated with a transitional metal chelator as described in the present invention. These results strengthened the surprising and unexpected results disclosed in Example 1. Specifically, the trials showed the superior expansion of not only Mononuclear Cells (MNC) (expansion from 74 to 420.5 fold) but also the striking

and superior selective expansion of CD34⁺ cells (expansion from 1.9 to 57.8 fold). More specifically, patient 1 showed a very good 2 fold expansion, patients 3 and 4 an excellent 20.9 and 29.6 fold expansion, respectively and patient 2 a dramatic 57.8 fold expansion of clonogenic cells (CFUc).

In sum, these results demonstrate that the claimed methods result in the superior expansion of clonogenic cells (CFUc), and that this in turn will result in greatly improved short-term and long-term engraftment effectiveness (*See, Appendix A*).

11. The present invention has also received praise in receiving the “Best Abstracts Award” from the American Society for Blood and Marrow Transplantation and has been praised in The Wall Street Journal health section for the improved long-term engraftment effectiveness of hematopoietic cells expanded by the process described in the claimed methods (*See, Appendix B*).
12. Moore and De Bruyn both refer to hematopoietic umbilical cord blood derived progenitor cells obtained from a donor. Neither Moore nor De Bruyn explicitly teach or suggest methods of transplanting hematopoietic cells or methods of adoptive immunotherapy by obtaining hematopoietic cells from a donor, providing the hematopoietic cells *ex vivo* with a transition metal chelator having an affinity for copper where the chelator and conditions result in (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state; thereby expanding the cells and transplanting the cells to a patient.
13. Cicuttine refers to the control of proliferation of stromal cell lines that are in turn co-cultured with certain hematopoietic cells – Zinc was added to the culture medium only to switch on the T oncogene under the control of a Zn-responsive element of a metallothionein promoter relating to the proliferation of the underlying stromal cell line (*See, Cicuttine at page 103, column 1, lines 25-28*). Moreover, the specified culture conditions exclude the possibility that the hematopoietic and stroma cell co-cultures are initiated in the presence of zinc as the zinc is specifically washed out prior to the introduction of hematopoietic cells to the co-culture (*See, Cicuttine at page 104, column 2, lines 7-12*). Cicuttine does not refer in any way to use of a

transition metal chelator with an affinity for copper in the culture of hematopoietic cells for (i) prolonged active cell proliferation; (ii) prolonged expansion of clonogenic cells (CFUc); and (iii) maintenance of undifferentiated cells in their undifferentiated state. The Examiner states that “zinc has an affinity to copper and thus would reduce copper utilization of culturing hematopoietic cells”. The Examiner concludes that “it would have been obvious to one of ordinary skill in the art ... that culturing the cell in the medium containing zinc would reduce a capacity of hematopoietic cells in utilizing copper”. *See*, Office Action at page 4, fifth paragraph. The Examiner is apparently taking the position that Zinc is a transition metal chelator with an affinity for copper. Zinc is a transition metal and not a transition metal chelator with an affinity for copper, as required by the claimed methods. Further, since both zinc and copper ions are positively charged species in solution, “affinity” is not likely. Moreover, as stated *supra*, the hematopoietic cells are not cultured in a medium containing zinc as suggested by the Examiner. Finally, there is no support in Cicuttine for the suggestion that zinc has an affinity for copper; in fact, the term copper is not disclosed in Cicuttine.

14. Percival I refers to a method of incubating of HL-60 cells with TEPA which results in reduced copper levels. Percival I does not teach or suggest “culturing conditions using defined growth medium conditions that will stimulate growth while inhibiting differentiation” (*See*, Office Action at page 5, first paragraph, lines 1-2) and that “chelating copper with TEPA will inhibit differentiation” (*See*, Office Action at page 5, first paragraph, lines 5-6) as suggested by the Examiner. Percival I is correctly interpreted to mean that TEPA had no effect on the differentiation of the single cell line – HL-60 cells – used in Percival I. Other statements in Percival I clearly support this interpretation. The authors of Percival I hypothesized that TEPA may be an inducer of differentiation, and specifically test TEPA to determine if TEPA acts as an inducer of HL-60 differentiation: “It was necessary ... to determine whether incubating the HL-60 cells with the copper-chelating compound would result in differentiation ... [i]ncubating cells with TEPA did not affect the respiratory burst activity, demonstrating that neither the compound nor the chelation of copper resulted in cell differentiation.” (*See*, Percival I at page 2428, column 1, lines 22-34).

15. I have also reviewed a later publication by Percival (Am. J. Clin. Nutr. 67:1064-68, 1998) (“Percival II”), which clarifies, comments on and expounds on the results of Percival I (noted as reference 27 in Percival II). Percival II is quite clear – it first demonstrates that “copper supplementation enhanced retinoic acid-induced differentiation [of HL-60 cells]” (*See, Percival II* at page 1066S, column 2, lines 5-6, referring to the studies of Bae and Percival, J. Nutrition 123: 997-1002 (1992)) and then based on that finding Percival II asks the following question: “If copper is removed from the cell is differentiation impaired or prevented?” (*See, Percival II* at page 1066S, column 2, lines 7-8). The author of Percival II hypothesizes, similarly as the Examiner, stating “[w]e hypothesized that if copper is essential for differentiation, then chelation of copper with TEPA should prevent the cells from differentiating.” (*See, Percival II* at page 1066S, column 2, lines 8-11). Percival II then cites the studies of Percival I showing that this hypothesis is incorrect stating, “Cells incubated with TEPA and retinoic acid produced the same amount of superoxide anion as did the cells with retinoic acid, **indicating that differentiation had occurred.**” (Emphasis Added) (*See, Percival II* at page 1066S, column 2, lines 15-18). Percival II further states that “So whereas our TEPA model is useful in some studies related to manipulating copper concentrations and Cu/Zn SOD activity, **it does not prevent HL-60 cells from differentiating.**” (Emphasis Added) (*See, Percival II* at page 1066S, column 2, lines 28-30). This teaches away. In fact, it teaches precisely the opposite result achieved with the claimed methods. In fact, the inability of TEPA to inhibit differentiation prompted the author to develop a different model to study copper’s role in the differentiation of HL-60 cells, “The lack of effect of TEPA on HL-60 differentiation prompted us to develop a mouse model to continue our investigation of copper’s role in granulopoiesis.” (*See, Percival II* at page 1066S, column 2, lines 31-33).

16. In reading Percival I and Percival II, it is clear that Percival I does not teach (i) prolonged active cell proliferation as Percival I states that for HL-60 cells, TEPA “did not affect the growth rate” – i.e., expansion was unaffected by a transition metal chelator and (ii) prolonged expansion of clonogenic cells (CFUc) as Percival I does not refer to this population of cells at all. Percival I refers only to HL-60 cells, whose expansion, unlike the claim recited population, was unaffected by a transition metal

chelator. HL-60 is an immortalized cell line, which proliferates continuously without undergoing terminal differentiation under normal culture conditions (such as provided in Percival I), unless supplemented with a specific differentiation inducer such as retinoic acid. Moreover, the HL-60 cell line of Percival I was derived from a patient with acute promyelocytic leukemia, cannot be isolated from a normal donor or another patient, nor can it be introduced into a human host because of its leukemic nature.

17. It is my view that the combination (and I do not believe this to be a proper combination) of Moore or De Bruyn with either Cicuttine or Percival I would not direct the ordinary skilled artisan to the unexpected results achieved with the claimed invention – namely prolonged active cell proliferation and prolonged expansion of a subset of hematopoietic cells, i.e. clonogenic cells (CFUs) -- and further would not direct the artisan to the unexpected, enhanced clonability of long term cultures. I am of the view that the present claimed methods of transplanting hematopoietic cells or methods of adoptive immunotherapy are not obvious in view of the cited art.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



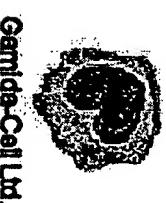
Prof. Eitan Fibach

Signed this day 14 of April, 2004

Enc.:
Appendix A and B

TRA 1902839v4

first 4 patients on GS trial IND 10751



APPENDIX A

Patient #	1	2	3	4
Diagnosis	AML	ALL	HD	ALL
Age	21	17	24	10
Gender	Female	Male	Male	Male
Weight (kg)	50	75.1	77	53
Cell source	Un. of Colorado	Milan	Un. of Colorado	Un. Of Colorado



Gamida-Cell Ltd.

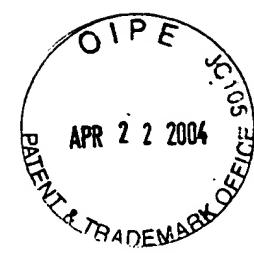
Units

Gamida-Cell Ltd.

APPENDIX A

	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4
Unit portions (%)	40/60	50/50	40/60	40/60
Viable MNCs in expanded portion ($\times 10^6$)	30	148	31	126
CD34+ cells in expanded portion ($\times 10^6$)	0.8	21.5	3.13	18
Fold expansion MNC	74	420.5	231	206
Fold expansion CD34	1.9	57.8	20.9	29.6





APPENDIX A

1.1.1.1 In-process data

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When the results of the endotoxin, sterility and mycoplasma tests are unacceptable, the manufacturing process is discontinued. The viability of the cells following selection with the CliniMACS should also meet specifications, however, proceeding with the manufacturing process is based on the transplanter medical judgment.

Test method	Specifications	Results*		
		Lot #BR004	Lot #BR005	Lot #BR006
Unit Portion (%)	-	40	.50	40
Number of viable MNCs thawed ($\times 10^6$)	-	792	1675	476.7
% Viability - post thaw	-	85.3	85.5	98.7
Number of AC133 ⁺ cells after column ($\times 10^4$)	-	42.5	37.2	15.4
% Viability - post CliniMACS	<50	54.8	17.4**	53.8
% Purity of CD133 ⁺ cells (CD133PE/CD38FITC)	-	65.8	94.8	73.4
CFU per 1000 cells	-	76	245	130
Endotoxin content (Eu/ml) Day=0		<2.0	<2.0	<2.0
Sterility-Bacteria Day=0	No growth	No growth	No growth	No growth
Sterility-Fungal Day=0	No growth	No growth	No growth	No growth
Mycoplasma	Absence	Absent	Absent	Absent
Sterility-Bacteria Day=7	No growth	No growth	No growth	No growth
Sterility-Fungal Day=7	No growth	No growth	No growth	No growth
Endotoxin content (Eu/ml) Day=14		<2.0	<2.0	<2.0

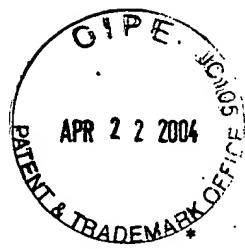
* The processing of the manufacturing data for the batch to be transplanted into patient #4 is not yet complete, and therefore unavailable at the deadline for the submission of this report.

** The transplanter instructed the technicians to place cells into culture and to measure viability by 7AAD flow cytometry. The result by this method was 76.2%.

1.1.1.2 Lot release data

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Test method	Specifications	Results*		
		Lot #BR004	Lot #BR005	Lot #BR006
Total number of viable cells ($\times 10^6$)	-	30	148	31
% Viability	-	100	94.9	100
Fold Expansion of cells	-	74	420.5	231.3
% of CD34 ⁺ cells	-	2.65	14.53	10.1
% of CD34 ⁺ /CD38 ⁻ cells	-	0	0.34	0.84
% of CD133 ⁺ cells	-	0.53	5.04	6.24
% of CD133 ⁺ /CD38 ⁻ cells	-	0	0.27	0
CFU per 1500 cells	-	25.7	39	35.3
Gram stain	Negative	Negative	Negative	Negative
Endotoxin content (Eu/Kg)	<5 Eu/Kg/60min	3.96	2.77	2.68
Sterility-Bacteria	No growth	No growth	No growth	No growth
Sterility-Fungal	No growth	No growth	No growth	No growth
Mycoplasma	Absence	Absent	Absent	Absent



APPENDIX A

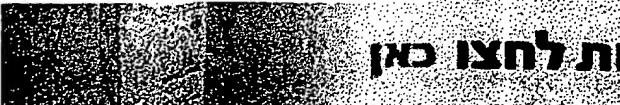
The processing of the manufacturing data for the batch to be transplanted into patient #4 is not yet complete, and therefore unavailable at the deadline for the submission of this report.

1.1.1.3 General lots details*

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	Patient #001	Patient #002	Patient #003
Lot number	BR004	BR005	BROO6
Cell source	University of Colorado Cord Blood Bank	Milan Cord Blood Bank (Rome)	University of Colorado Cord Blood Bank
Start of Manufacturing	March 26, 2003	April 10, 2003	July 22, 2003
End of Manufacturing	Apr. 16, 2003	May 1, 2003	Aug 12, 2003
Patient administration	Apr. 16, 2003	May 1, 2003	Aug 12, 2003

* The processing of the manufacturing data for the batch to be transplanted into patient #4 is not yet complete, and therefore unavailable at the deadline for the submission of this report.



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Thursday February 19, 2004

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Israeli start-up Gamida-Cell to receive prize

Gamida-Cell, which is active in the field of blood stem cell expansion to treat disease, has raised \$20 million to date.

Batya Feldman — 5 Feb 04 16:54

Israeli start-up Gamida-Cell has been chosen to receive the "Best Abstracts Award" by the American Society for Blood and Marrow Transplantation. The award will be presented to the Gamida-Cell research team on February 13th in Orlando, Florida.

Gamida-Cell is active in the field of blood stem cell expansion for cancer and autoimmune disease therapies. The Gamida-Cell abstract describes test results in which the injection of expanded populations of human stem cells restored cardiac function in animals that had suffered a heart attack. Because of these results, the company has fast tracked plans to commence a Phase I/II study in 2004.

Gamida-Cell was founded in 1998. Since then, the company has raised \$20 million in three rounds of financing. Shareholders include Mordechai (Motti) Zisser, Biomedical Investments, [Teva Pharmaceuticals](#) (Nasdaq:TEVA; TASE:TEVA), and venture capital funds Denali Ventures, Auriga Ventures, Pamot Rehovot Advisers' Pamot Venture Capital fund and Converse subsidiary Comsor Venture Fund.

Gamida-Cell CEO Ehud Marom, a veteran in the healthcare field, is a former VP of Teva and Peptor, and one of the founders of Karma Pharm, which was acquired for \$2 million by Biodar Pharma last month. Marom told "Globes" that last March, Gamida-Cell signed a strategic co-operation agreement with Teva (Nasdaq: TEVA). Under the agreement, Teva invested \$3 million in Gamida-Cell, with an option for further investment once clinical trials were completed. Marom said this investment would be about \$25 million, plus a 15% stake in the company.

Marom said that the company was now in a much better position to raise capital and sign strategic agreements. In the coming year, he said, Gamida-Cell would complete the first two phases of clinical trials, the goal being to realize the agreement with Teva. Marom also did not rule out the possibility of the company being acquired by a large corporation. He believes that if the market continues to be positive, major companies will seek out acquisitions. Gamida-Cell was certainly a good candidate, he said.

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APPENDIX B

HEALTH

FROM THE ARCHIVES: June 12, 2003

Stem-Cell Technology to Treat Leukemia Patients Shows Promise

By DAVID P. HAMILTON
Staff Reporter of THE WALL STREET JOURNAL

A phase I trial of StemEx, a technology from Gamida-Cell Ltd. for expanding populations of so-called hematopoietic stem cells, is off to a good start, the company's chief executive said in an interview.

"The results are encouraging," said Gamida-Cell CEO Ehud Marom, without providing details. "It's a beginning, a good beginning."

Gamida-Cell, a closely held biotechnology firm based in Jerusalem, Israel, is testing StemEx in conjunction with the M. D. Anderson Cancer Center at the University of Texas in Houston.

The goal, Mr. Marom said, is to use StemEx to expand populations of so-called hematopoietic stem cells from stored umbilical-cord blood in order to reconstitute the immune systems of patients with leukemia or other blood cancers.

The process is similar to that involved in a bone-marrow transplant. In that procedure, doctors use chemotherapy and radiation to deliberately destroy the blood cells and immune system of leukemia patients, whose blood cells are multiplying out of control. Then an immunologically matched donor provides bone marrow containing hematopoietic stem cells, which can grow into a variety of different blood cells. When provided to the cancer patient, the cells reconstitute the patient's immune system, minus the cancer.

In this trial, physicians hope to use stem cells from cord blood, which some parents have stored shortly after the birth of their children, for those transplants. Cord blood contains a population of young stem cells that are thought to be particularly useful in transplantation.

Normally, however, cord blood doesn't yield enough stem cells to treat adult cancer patients. So doctors in the Houston trial will use StemEx, a chemical formulation that encourages the proliferation of stem cells, to "expand" populations of stem cells

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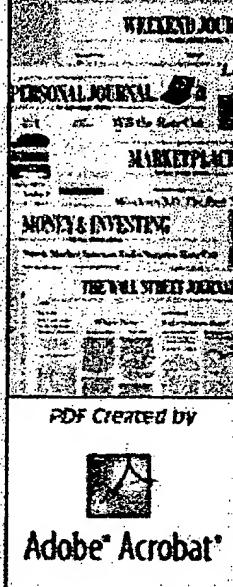
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by as much as 100 to 1,000 times in culture. Animal studies have shown StemE-~~ce~~ in not only expanding stem-cell populations, but in producing better "grafts" leading to reconstituted immune systems, Gamida Cell says.

The study should involve 10 patients, all over 55 years old, with a variety of blood cancers. Mr. Marom said the company hopes to have recruited all 10 patients by the end of the year. If the trial goes well and the U.S. Food and Drug Administration approves, Mr. Marom said, the company hopes to move quickly to a larger, multi-center trial of about 80 patients.

Write to David P. Hamilton at david.hamilton@wsj.com

Updated June 12, 2003

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